BABA GHULAM SHAH BADSHSH UNIVERSITY
RAJOURI

SYNOPSIS FOR Ph.D REGISTRATION

Proposed Research Topic	:	Studies on taxonomy and ecology of Rhabditid nematodes of upper and lower climatic zones of district Poonch
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Subject	:	Zoology
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Signature of the Candidate **Signature of the Supervisor**

Background:

Nematodes inhabit virtually all ecosystems. They are part of phylum Nemata that includes plant, insect, animal and human parasites and predatory forms that consume microorganisms including bacteria, fungi, algae and other nematodes. In general nematodes are small, usually less than a millimeter in size. Some animal parasitic nematodes are, however, large and can be seen by the naked eye Wallace *et al.,* (1996). Several nematode genera are important as pests of crops and animals while others contribute to nutrient mineralization Ferris and Matute (2003) and control of plant pests and pathogens Grewal *et al.,* (2005).

The earliest written account of a nematode sighting is found in the Pentateuch of the Old Testament in the Bible. Borellus (1653) was the first to observe and describe a free-living nematode, which he dubbed the "vinegar eel," and Tyson (1683) used a crude microscope to describe the rough anatomy of the human intestinal roundworm, *Ascaris lumbricoide*s. Needham (1743) was the first to observe a plant parasitic nematode from crushed diseased wheat grains.

As far as free-living nematodes are concerned, Dujardin (1845) was the first to establish the genus *Rhabditis* with *Rhabditis terricola* as its type species. However, it was not clearly defined until more than one hundred years later (Dougherty, 1955). Orley (1880) tried to fit the genus *Rhabditis* into the system of Nematoda and proposed a family Rhabditidae for the genera *Anguillula*, *Cephalobus*, *Oxyuris*, *Rhabditis* and *Teratocephalus* and placed this family in the higher category ‘Rhabdidiformae which formed a connecting link between free-living and animal- parasitic nematodes. He provided new information regarding biology and taxonomy of Rhabditis besides a key for the identification of species. De Man (1876, 1880, 1884) added many new species with thirty-seven species in *Rhabditis*. Micoletzky (1922) in his monograph on the non-marine free-living nematodes listed 142 valid genera and 931 species. Cobb (1913)

proposed the genus *Diploscapter* for *Rhabditis coronata*. Goodey (1963) accepted four subfamilies: Alloionematinae, Protorhabditinae, Diploscapterinae and Rhabditinae under Rhabditidae and also proposed Pterygorhabditinae under Bunonematidae. He also accepted Bunonematinae Micoletzky (1922). Andrassy (1976) accepted three superfamilies viz., Allonematoidea Chitwood & McIntosh (1934), Rhabditoidea Orley (1880) and Bunonematoidea Micoletzky (1922) under Rhabditina. Under Rhabditidae, he proposed seven subfamilies including three new ones viz., Mesorhabditinae, Peloderinae and Ablechroiulinae and upgraded the subfamily Pterygorhabditinae to family Pterygorhabditidae under Bunonematoidea.

Nematological research in India has mostly been focused on the plant and animal parasitic groups. Little has been done on the nematodes belonging to free-living groups, probably because they have no direct concern with agriculture and livestock. As soil microorganisms, however, they play an important role in ecology. Most of the work was done during the late 60s and 70s and only few scientists worked on these groups. The earliest report is the description of the genus *Tridontus longicaudatus* Khera (1965) (*Monochoides longicaudatus*). Later Khera (1969) described *Mesodiplogasteroides* and in 1970 *Paradoxogaster* (*Anchidiplogaster*) and *Gobindonema* (*Koerneria*). *Tawdemma* was described by Suryawanshi (1971) and was synonymised with *Acrostichus* and *Syedella* was also synonymised with *Pareudiplogaster*. *Tridontus longicaudatus* was redescribed by Jairajpuri *et al.* (1973) and they synonymised *Syedella* with *Tridontus*. Khera (1969) described the genera *Saprorhabditis* in Protorhabditinae (now in Rhabditonematinae), *Praeputirhabditis* (*Cuticularia*) in Rhabditinae and *Operculorhabditis* in Rhabditinae (now in Mesorhabditinae) and 1971 *Paradoxorhabditis* in Rhabditinae (now in Protorhabditinae). Tahseen and Jairajpuri (1988) described *Teratorhabditis*

andrassyi and its biology. Huseni *et al.,* (1997) described three species *Bunonema irregularis*, *Bunonema minutum* and *Pterygorhabditis superbus* in Superfamily Bunonematoidea.

In the past 20 years, considerable attention has been paid to soil nematodes, demonstrating that these ubiquitous members of the soil community reflect change in ecological structure and function of soils in ways more predictable and efficient than for other soil flora or fauna. The free-living nematodes are very important and beneficial in the decomposition of organic matter and the recycling of nutrients in soil. These nematodes have been evaluated for their use as indicators due to several attributes they possess. Species richness is most commonly used indicator but it is highly dependent on sampling efforts. They are frequently the most numerous component of the microfauna and are considered as the most abundant and diverse invertebrates present in the soil (Yeates, 1979). Although, nematodes represent a relatively small amount of biomass in the soil, their occurrence across multiple trophic levels is vitally important in the soil environment (Barker and Koenning, 1998). Since their feeding habits are clearly related to oral structure, their trophic roles are readily inferred. Each soil sample contains an abundance and diversity of nematodes and, consequently, has high intrinsic information value (Bongers and Ferris, 1999; Yeates *et al.,* 1993). In addition to this, nematodes are easy to extract from the soil using simple extraction procedures (Ritz and Trudgill, 1999).

Bongers (1990) classified nematodes along a colonizer-persister (c-p) continuum of 1-5. Nematodes with c-p value equal to one are short lived, have high fecundity, feed on enriched media whereas those of c-p value five have large body size, longer life span, low fecundity, susceptible to disturbance and are predominantly omnivores and predators (Bongers, 1990). C-p classifications of nematodes lead to the formation of the maturity index (MI), which is a

weighted mean frequency of c-p scaling across the entire nematode community and provides information of the likely condition of the soil environment (Bongers, 1990). The development of MI represented a significant advancement in interpreting the relationships between the ecology of nematode communities and functions of the soil (Neher *et. al.,* 2005). Calculation of the MI index also assumes a progression of soil conditions from stressed or polluted to pristine exactly congruent with the continuum of nematode life history characteristics in the c-p classification as suggested by Bongers, (1990). Ferris *et. al.,* (2001) observed that the most abundant nematode taxa under stressed conditions are those in c-p 2, while the enrichment opportunists c-p 1 respond positively to disturbances that result in enrichment of the food web.

In India extraordinary species diversity of nematodes and the paucity of trained nematode taxonomists for species-level identification have challenged nematode taxonomy. Without the ability to easily and efficiently discriminate among species, nematodes will remain excluded from many areas of biological research.

Morphology has been and will continue to be the mainstay of nematode taxonomy. It is an essential component of any higher level classification of nematodes and in many cases morphology provides a rapid and unambiguous diagnosis to species. However, the identification of intrasubspecific groupings requires the application of other taxonomic techniques such as DNA sequence analysis. In view of the above DNA-based taxonomic methods will be used for the identification of nematode species wherever required.

The present plan of work is proposed to study the taxonomy and ecology of Rhabditid nematodes of upper and lower climatic zones of district Poonch.

Objectives of the study:

- Proper Inventorising of Rhabditid nematodes of district Poonch.
- Description and redescription of all the taxa isolated.
- Correlate the taxa isolated with their ecological conditions.
- Molecular characterization of the isolated taxa using RAPD markers wherever required.
- To check the organic and inorganic contents of the collected soil samples of district Poonch.

Methodology

(a) Methodology for Taxonomic Study:

Classical Taxonomy

For morphological identification the methodology involve following steps:-

Isolation of nematodes: Nematodes will be isolated by using modified Cobb's sieving and decantation and modified Baermann's funnel technique.

Killing and fixation of nematodes: The nematodes will be killed and fixed in hot FA 4:1 (Seinhorst, 1962).

Dehydration, mounting and sealing: After fixation the nematodes will be transferred into a mixture of glycerine-alcohol (5 parts glycerine + 95 parts 30% alcohol). Gradual dehydration will be done by using anhydrous calcium chloride. Mounting will be done in anhydrous glycerine and subsequent sealing by wax.

Measurements and drawings: The measurements will be taken by using an ocular micrometer and dimensions will be expressed by using De Man's formula. The illustrations will be drawn with the help of a drawing tube attached to Olympus microscopes.

Photography: Photographs will be taken by using a digital camera attached to Olympus microscope.

Molecular Taxonomy

Nematodes will be cultured and maintained in agar medium till their final use. Nematode extraction buffer (NEB) method proposed by Z. A. Handoo *et.al.*, with modifications wherever required shall be followed for genomic DNA isolation. After purification, the extracted worm DNA shall be subjected to RAPD marker analysis.

(b) Methodology for ecological study:

Sampling: Soil samples will be collected in different seasons from upper and lower climatic zones of district Poonch to isolate nematodes of the order Rhabditida. Soil sampling will be done according to Southy, (1974) sampling method. The samples will be kept in air tight polythene bags. All relevant information such as locality, date of collection will be noted and the samples brought to the laboratory for further processing.

Study of soil:

Soil pH: The pH will be determined using pH meter on a 1:1 suspension (5 gram scoop of soil to 5 milliliters water).

Soil moisture: The moisture content of the samples will be determined by oven-drying method.

Soil organic matter: Organic matter will be determined by ashing a 5 gram scoop of the soil sample at 360^{o}C for 2 hours. The loss by weight of the sample during this ignition is calculated as the organic matter.

Nematode enumerations: Nematodes will be counted, heat relaxed, fixed in hot F.A. (4:1), and mounted on temporary slides for identification of at least 10% or upto 150 representative individuals per sample to genus level according to Thorne (1974), Jairajpuri and Ahmad (1992), Bongers (1994), Siddique (2000) and De Ley *et al.,* (2003). Nematodes will be assigned trophic groups according to Yeates *et al.,* (1993) and colonizer- persister groups based on Bongers (1990).

Community indices: Nematode communities will be characterized using ecological indices. Generic richness defined as the total number of genera observed per sample. Hill's diversity index will be calculated as $N1 = \exp(-\sum p_1 \ln(p_1=0.001)$ where p_1 is the proportion of each genus i (Hill, 1973). Maturity index (Bongers,1990), a measure of nematode community succession, will be calculated as $MI=\sum (v_i p_i)$, where v_i, is the colonizer-persister (c-p) value assigned to free- living (not herbivorous) taxa I, and pi is the propotion of Taxon I (Bongers, 1990).

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(* not seen in original)